Heredity (2017) 119, 411–417

www.nature.com/hdy

ORIGINAL ARTICLE

The effects of outbreeding on a parasitoid wasp fixed for infection with a parthenogenesis-inducing *Wolbachia* symbiont

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Trichogramma wasps can be rendered asexual by infection with the maternally inherited symbiont *Wolbachia*. Previous studies indicate the *Wolbachia* strains infecting *Trichogramma* wasps are host-specific, inferred by failed horizontal transfer of *Wolbachia* to novel *Trichogramma* hosts. Additionally, *Trichogramma* can become dependent upon their *Wolbachia* infection for the production of female offspring, leaving them irreversibly asexual, further linking host and symbiont. We hypothesized *Wolbachia* strains infecting irreversibly asexual, resistant to horizontal transfer *Trichogramma* would show adaptation to a particular host genetic background. To test this, we mated *Wolbachia*-dependent females with males from a *Wolbachia*-naïve population to create heterozygous wasps. We measured sex ratios and fecundity, a proxy for *Wolbachia* fitness, produced by heterozygous wasps, and by their recombinant offspring. We find a heterozygote advantage, resulting in higher fitness for *Wolbachia*, as wasps will produce more offspring without any reduction in the proportion of females. While recombinant wasps did not differ in total fecundity after 10 days, recombinants produced fewer offspring early on, leading to an increased female-biased sex ratio for the whole brood. Despite the previously identified barriers to horizontal transfer of *Wolbachia* to and from *Trichogramma pretiosum*, there were no apparent barriers for *Wolbachia* to induce parthenogenesis in these non-native backgrounds. This is likely due to the route of infection being introgression rather than horizontal transfer, and possibly the co-evolution of *Wolbachia* with the mitochondria rather than the nuclear genome. These results help to elucidate the mechanisms by which *Wolbachia* adapt to hosts and the evolution of host-symbiont phenotypes.
Heredity (2017) **119,** 411–417; doi:10.1038/hdy.2017.53; published online 13 September 2017

INTRODUCTION

Wolbachia is a maternally inherited symbiont of arthropods and nematodes that is estimated to infect between one and five million species (Werren and Windsor, 2000). The success of *Wolbachia* is attributed to its ability to increase host fitness through a variety of mechanisms, including reproductive modifications such as cytoplasmic incompatibility, male-killing, feminization, and parthenogenesis-induction (Werren *et al.*, 2008). These manipulations increase the relative fitness of infected females within a population. As such, *Wolbachia* has the ability to invade populations, and the infection can become fixed (Turelli and Hoffmann, 1991; Stouthamer *et al.*, 2010). In certain cases, the host can become dependent on its resident *Wolbachia* infection after fixation in the population, and removal of the symbiont can result in severe fertility reduction (Hoerauf *et al.*, 1999; Dedeine *et al.*, 2001; Stouthamer and Mak, 2002; Kremer *et al.*, 2009; Hosokawa *et al.*, 2010; Russell and Stouthamer, 2011).

Dependence upon *Wolbachia* infection has been documented several times in hosts for parthenogenesis-inducing *Wolbachia* (Dedeine *et al.*, 2001; Stouthamer and Mak, 2002; Kremer *et al.*, 2009; Russell and Stouthamer, 2011). Parthenogenesis-inducing *Wolbachia* have been described in wasps, thrips, and mites: all arthropods with haplodiploid sex determination (Werren *et al.*, 2008). Across *Wolbachia*, there are several independent transitions to the parthenogenesis-inducing phenotype, as well as horizontal transfer of parthenogenesis-inducing *Wolbachia* between hosts (Stouthamer *et al.*, 1993; Werren *et al.*, 1995; Schilthuizen and Stouthamer, 1997). Horizontal transfer of parthenogenesis-inducing *Wolbachia* has been documented in the lab between parasitoid wasps that superparasitize the same host egg (Huigens *et al.*, 2000, 2004). Once infected, hosts that normally produce via arrhentoky (males develop from unfertilized eggs) are converted to thelytokous reproduction (females developed from unfertilized eggs). The transition to thelytokous reproduction aids *Wolbachia* in driving through the population, after which the host can develop dependence upon its *Wolbachia* symbiont (Stouthamer *et al.*, 2010).

This phenomenon of symbiont-dependence has been documented in some populations of *Trichogramma* parasitoid wasps. There is extensive inter- and intra-specific variation in *Wolbachia* infection frequencies across *Trichogramma*: some populations are never found with *Wolbachia* (Pinto, 1998), others maintain a consistent low-level infection (Stouthamer and Kazmer, 1994; Stouthamer *et al.*, 2001), and some are completely fixed for *Wolbachia*-infection (Russell and Stouthamer, 2011; Almeida and Stouthamer, 2015). The extremes of this variation are particularly evident in one species, *Trichogramma pretiosum*. *Wolbachia* appears to be absent from wild populations of *Trichogramma pretiosum* in California (Pinto, 1998), but in contrast,

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Received 14 June 2017; revised 8 August 2017; accepted 9 August 2017; published online 13 September 2017

some conspecific populations elsewhere are fixed for infection, and have evolved complete dependency upon *Wolbachia*-induced thelytokous parthenogenesis for the production of female offspring (Stouthamer *et al.*, 2010; Russell and Stouthamer, 2011).

This dependence results from the mechanism by which *Wolbachia* manipulates the haplodiploid sex determination system of *Trichogramma*. *Wolbachia* duplicates chromosomes in unfertilized *Trichogramma* eggs, turning what would have been a haploid male offspring into a diploid female offspring. The duplication occurs through a failed anaphase during the first mitotic division of the egg (Stouthamer and Kazmer, 1994). Over time, *Trichogramma* become dependent on this gamete duplication for the production of female offspring. If cured of their *Wolbachia* infection, eggs are not fertilized at high enough rates (ensuring females) to sustain the population (Jeong and Stouthamer, 2005; Stouthamer *et al.*, 2010; Russell and Stouthamer, 2011). As such, irreversibly asexual *Trichogramma* are now tied to their resident *Wolbachia* infection.

While *Wolbachia*'s primary form of transmission is vertical, from mother to offspring, over evolutionary time horizontal transfer across species appears to have been common (Schilthuizen and Stouthamer, 1997; Zhou *et al.*, 1998; Stouthamer *et al.*, 1999a; Vavre *et al.*, 1999). That said, experimental attempts at horizontal transfer between *Trichogramma* wasps have proved largely unsuccessful, resulting in the loss of bacterial titers or failure to express parthenogenesis (Grenier *et al.*, 1998; Pintureau *et al.*, 2000; Huigens *et al.*, 2004). This is surprising given that the *Wolbachia* strains infecting *Trichogramma* wasps are closely related to each other (Werren *et al.*, 1995; Schilthuizen and Stouthamer, 1997), and that horizontal transfers across larger phylogenetic distances are often successful with other *Wolbachia* strains and other insect hosts (Heath *et al.*, 1999; Kang *et al.*, 2003; Hoffmann *et al.*, 2011). The exception to this pattern is horizontal transfers of *Wolbachia* between *Trichogramma* lines that originate from mixed-infection populations (Huigens *et al.*, 2000, 2004). *Trichogramma kaykai* is well documented for maintaining mixed infections within a region (Pinto *et al.*, 1997; Huigens *et al.*, 2004), which is likely behind the propensity of such *Wolbachia* strains to successfully establish in other *Trichogramma* hosts (Huigens *et al.*, 2000, 2004). In such environments, it would be beneficial for *Wolbachia* to readily transfer to new hosts, as the chance of *Wolbachia* encountering an uninfected individual would be quite high. This is in contrast to regions of fixed infection across the population where *Wolbachia* would need to compete with already established infections in any new host they encounter. As horizontal transfers become increasingly rare, *Wolbachia* would become linked to a host genetic background. In contrast to the mixed-infection status and frequent horizontal transfers amongst *Trichogramma kaykai*, *Trichogramma pretiosum* is one such species where infection status is often fixed across a region (Pinto, 1998; Almeida and Stouthamer, 2015), and horizontal transfers in the lab have failed (Huigens *et al.*, 2004).

It was hypothesized that the failed transfers amongst these wasps are due to a lack of co-evolution between *Wolbachia* and the novel *Trichogramma* host (Huigens *et al.*, 2004; Watanabe *et al.*, 2013). Here, we test this prediction by manipulating the nuclear genetic background of an irreversibly asexual line originating from a region with complete infection for *Wolbachia*, and observing how *Wolbachia* performs in the new genetic background. We can manipulate the genetic background of *Trichogramma* by exploiting the fact that some irreversibly asexual *Trichogramma* will, if mated to males derived from sexual populations, fertilize a small proportion of their eggs (Stouthamer and Kazmer, 1994), resulting in a small number of heterozygous offspring. Heterozygous daughters of this cross will then produce recombinant *Wolbachia*-infected daughters that can be used to start a unique colony, in which gamete duplication renders all subsequent offspring identical and homozygous (Figure 1a). The newly recombined host-alleles allow us to test whether or not *Wolbachia* is adapted to a particular *Trichogramma* host genome.

MATERIALS AND METHODS

Trichogramma colonies

Two separate lines of *Trichogramma pretiosum* were used in experiments. The first, referred to as the 'Insectary line', was originally collected from the Piura Valley of Peru in 1966, and has since been continuously maintained in a commercial insectary (Beneficial Insectary, Guelph, Ontario, Canada). The

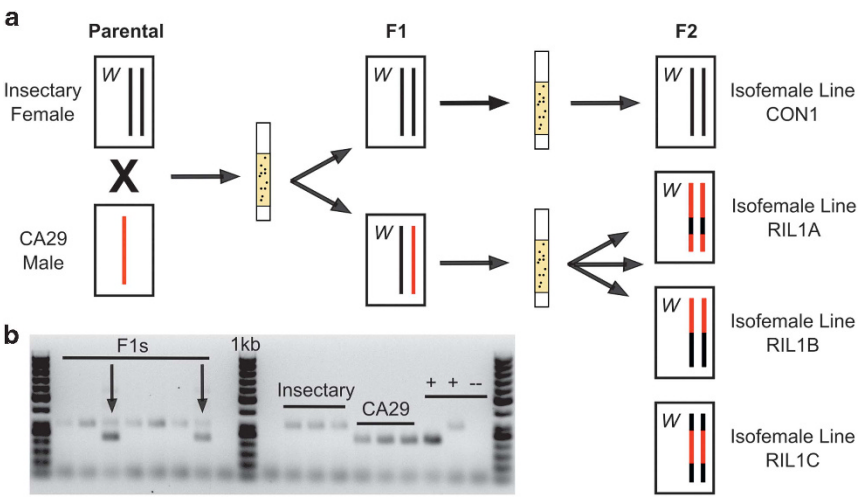

Figure 1 Experimental design for creating recombinant and control lines. (**a**) Crossing scheme. '*W*' indicates infection with *Wolbachia*, inherited maternally. Yellow bars with black dots represent parasitized egg cards. F1 offspring were screened for zygosity while F2s develop. (**b**) Microsatellite PCR assay for determining zygosity. Arrows on the gel image point to heterozygote F1s, from which individual offspring were isolated to initiate Recombinant Isofemale Lines (RILs). Positive controls are previously amplified samples from the Insectary and CA-29 lines, where as the Insectary and CA-29 labels indicate exact parents used in crosses.

Insectary line was described previously (Lindsey *et al.*, 2016; Lindsey and Stouthamer, 2017) and exhibits thelytokous parthenogenesis with females hatching from unfertilized eggs as a result of infection with *Wolbachia*. All *Trichogramma pretiosum* from Peru that have been tested for *Wolbachia* are infected and reproduce via thelytokous parthenogenesis (Russell and Stouthamer, 2011; Almeida and Stouthamer, 2015). The Insectary line, while it cannot be converted to a self-sustaining sexual line through antibiotic curing, will fertilize enough eggs to perform introgressions. This is in contrast to other isofemale lines of *Trichogramma pretiosum* that are unable to fertilize any of their eggs, preventing us from using them for introgression (Russell and Stouthamer, 2011). Additionally, because the Insectary line has been isolated as a clonal culture for more than 50 years (~1650 generations), it further ensures that there has been no novel genetic material introduced in quite some time, and *Wolbachia* had the opportunity to adapt to the host background. The second line, CA-29, is a highly inbred line initiated from a sib-mated female collected in Irvine, California in 2008. CA-29 exhibits normal haplodiploid sex determination (arrhenotokous parthenogenesis) with males developing from unfertilized eggs, indicating no parthenogenesis-inducing *Wolbachia*. No *Wolbachia*-infected *Trichogramma pretiosum* have been collected from this region, providing a genetic background that is not co-evolved with *Wolbachia* (Pinto, 1998). These two lines are ~1% diverged from each other, genome wide (Lindsey *et al.*, unpublished results). Infection status was confirmed by PCR assays following Werren and Windsor (2000). Species identifications were confirmed by molecular protocols from Stouthamer *et al.* (1999b). Colonies are maintained in 12×75 mm glass culture tubes stopped with cotton and incubated at 24 °C, *L*:*D* = 16:8. Every 11 days, colonies are given honey and new hosts: a surplus of UV irradiated *Ephestia kuehniella* host eggs (Beneficial Insectary, Guelph, Ontario, Canada) affixed to card stock with double-sided tape (egg cards).

Crossing experiments

We set up crosses of *Wolbachia*-infected and *Wolbachia*-uninfected lines to look for evidence of co-adaptation between *Wolbachia* and host that breaks down when genomes are recombined, and *Wolbachia* is inherited from the mother. Eight replicate crosses of a virgin female from the Insectary line with a virgin male from CA-29 were performed (F0). After copulation, females were allowed to parasitize an egg card for 48 h, after which they were removed and killed in 100% ethanol. Under this mating regime, the female can produce both homozygous F1 daughters (from unfertilized eggs that undergo *Wolbachia*-induced gamete duplication), and heterozygous F1 daughters (from eggs fertilized with sperm from the CA-29 male). Figure 1a shows the set-up of this crossing experiment. All F1 offspring were isolated as pupae to ensure virgin status, and upon emergence, each daughter was moved to a fresh egg card every 48 h for 10 days. At the end of this period, each daughter (F1 mother) was removed, killed in 100% ethanol, and her zygosity (either homozygous or heterozygous) was determined as described below (Figure 1b). Egg cards were maintained under colony conditions awaiting offspring emergence.

After zygosity was determined, F2 wasps were isolated as pupae from the first egg card of both heterozygous and homozygous F1's. Each female F2 from a heterozygous mother is: (1) a unique mix of the Insectary and CA-29 nuclear backgrounds as a result of recombination during meiosis; (2) infected with *Wolbachia* inherited from the Insectary line; and, (3) completely homozygous (because of gamete duplication). Three such F2 females from each of five parental (F0) crosses were used to initiate a total of 15 Recombinant Isofemale Lines (RILs). Five F2 wasps from homozygous F1 mothers (from the same F1 broods used for initiating RILs) were used to initiate control isofemale lines (CONs). Remaining F2 offspring produced throughout the 10-day trial were allowed to hatch, then were counted, and identified as female, male or intersex. Intersex *Trichogramma* are a result of incomplete feminization after gamete duplication (Tulgetske, 2010; Tulgetske and Stouthamer, 2012).

Experimental isofemale lines (RILs and CONs) were maintained under colony conditions, then after three generations they were assayed for sex ratio (hereinafter reported as the proportion of female offspring). Eighteen replicate wasps from each isofemale line were moved to fresh individual egg cards every 48 h for ten days, and subsequent offspring were counted upon emergence and identified as female, male or intersex based on antennal morphology. In summary, eight separate crosses were performed to generate heterozygote and homozygote wasps. We then randomly selected five of those crosses to initiate RILs and CONs.

Determination of zygosity

DNA was extracted from ethanol-preserved F1 mothers using the EDNA HiSpEx Tissue Kit (Fisher biotic, Australia). Zygosity was determined using a single microsatellite locus, arbitrarily designated A9. A PCR assay of the A9 locus, developed for use in our lab by Genetic Identification Services (Chatsworth, CA, USA), results in the production of a size-diagnostic amplicon: 310 bp for the Insectary line versus 220 bp for CA-29. Thus, in our crosses between Insectary females and CA-29 males, homozygous daughters (from unfertilized eggs that undergo *Wolbachia*-induced gamete duplication) are expected to produce a single 310 bp amplicon, while heterozygous daughters (from eggs fertilized with sperm from the CA-29 male) will produce both amplicons (Figure 1b). Reactions were set up in 25 μl volumes containing 1× ThermoPol buffer (New England Biolabs, Ipswich, MA, USA), 400 nM uracil, 200 nM each adenine, cytosine and guanine, 0.8 mM $MgCl_2$, 0.8 mg ml^{-1} BSA, 0.2 μM each of the primers A9F (5′-CAGCACAAGTACACGACTGTC-3′) and A9R (5′-AGCGAAGCGTATATTAGCAAG-3′), one unit *Taq* polymerase (New England Biolabs, Ipswich, MA, USA), and 2 μl DNA template. Reaction conditions were: an initial hold for 3 min at 94 °C, followed by 38 cycles of 94 °C for 45 s, 51 °C for 45 s, and 72 °C for 45 s. Reactions were terminated with a 5 min hold at 72 °C after which, PCR products were electrophoresed on 1.5% agarose gels stained with ethidium bromide.

Statistics

All statistics were performed in R version 3.3.2. We assessed variation in sex ratios and fecundity with generalized linear mixed-effects models (GLMM), using the glmer function from the lme4 package (Bates *et al.*, 2014), a Poisson error distribution for fecundity analyses, and a binomial (logit) error distribution for sex ratios. We used the number of females (successful parthenogenesis-induction events), and the number of non-female offspring (males plus intersex) as the response variable for the sex ratio analyses. Differences in survivorship, as measured by the number of wasps of each genotype that did and did not survive the length of the trial, were determined with a χ^2-test.

For comparisons among F1s, we assessed variation in total sex ratios or fecundity after the 10-day period (as described above) with genotype (either heterozygote vs homozygote) as a fixed effect, and initial F0 cross as a random effect. F1 sex ratios and fecundity over time (sex ratio or fecundity per egg card) were assessed with genotype and egg card number (time within trial) as fixed effects, along with their interaction, and individual wasp nested within initial F0 cross as a random effect.

For comparisons of RIL and CON reproduction, we assessed variation in total sex ratios or fecundity after the 10-day period with genotype (either RIL or CON) as a fixed effect, and unique line (for example, RIL3B or CON5) nested with initial F0 cross as a random effect. RIL/CON sex ratio and fecundity over time (sex ratio or fecundity per egg card) were assessed with genotype and egg card number as fixed effects, along with their interaction, and individual wasp nested within unique line, nested within initial F0 cross as a random effect. Correlation between sex ratio and 48 h fecundity was tested with a Spearman's rank correlation.

RESULTS

Heterozygous *Trichogramma* have higher fecundity, with no impact on total sex ratio

We created heterozygous and homozygous *Wolbachia*-infected *Trichogramma* wasps and assayed them for fecundity and offspring sex ratio over a ten-day period. 31.5% (24/76) of eggs were fertilized by the mated Insectary line mothers, resulting in heterozygotes. This is in contrast to sexual lines of *Trichogramma* that fertilize between 60 and 90% of their eggs (Suzuki *et al.*, 1984; Russell and Stouthamer, 2011), reaffirming that the Insectary line has significantly impaired sexual

function associated with the fixation of *Wolbachia* infection. Zygosity had no effect on the likelihood of wasps to survive the duration of the trial ($\chi^2 = 1.048$, $P = 0.3059$). We found a significant effect of the interaction between zygosity and time on sex ratios (Figure 2a; $\chi^2 = 165.5$, $P < 0.0001$). Wasp zygosity alone had a significant effect on sex ratios for each card (Figure 2a; $\chi^2 = 23.69$, $P < 0.0001$), as did time (Figure 2a; $\chi^2 = 1080$, $P < 0.0001$). Homozygous wasps showed a more drastic change in sex ratio with a particularly low level of parthenogenesis-induction for egg cards three and four (corresponding to trial days five through eight; Figure 2a). However, sex ratios of the entire 10-day brood were not significantly different between heterozygous and homozygous wasps (Figure 2b; $\chi^2 = 3.744$, $P < 0.5301$).

Similarly, we found a significant effect of the interaction between zygosity and time on fecundity across incremental 48 h periods (Figure 2c; $\chi^2 = 473.8$, $P < 0.0001$). Again, wasp zygosity alone had a significant effect on fecundity (Figure 2c; $\chi^2 = 14.40$, $P < 0.0001$), as did time (Figure 2c; $\chi^2 = 2192$, $P < 0.0001$). During the first 48 h period the control homozygous wasps produced more offspring than the heterozygotes but for the remainder of the trial, the heterozygous wasps out-performed the controls (Figure 2c). In contrast to there being no significant difference between total sex ratios produced by heterozygous and homozygous wasps, there was a significant difference in total fecundity after the 10-day period (Figure 2d; $\chi^2 = 116.9$, $P < 0.0001$).

Recombinant *Trichogramma* produce more female-biased sex ratios with no impact on total fecundity

We took offspring from virgin heterozygous *Trichogramma*, and used them to initiate RILs (F2). Similarly, we used offspring from homozygous *Trichogramma* to initiate control (CON) lines (F2). After allowing RIL and CON colonies to propagate for three generations, we assayed fecundity and sex ratio. RIL and CON wasps were equally as likely to survive the duration of the 10-day trial ($\chi^2 = 2.431$, $P = 0.1189$). There was a significant effect of the interaction between genotype (RIL or CON) and time on the sex ratios produced across incremental 48 h periods (Figure 3a; $\chi^2 = 85.00$, $P < 0.0001$). Likewise, sex ratio was significantly affected by genotype (Figure 3a; $\chi^2 = 10.82$, $P = 0.0010$), and time (Figure 3a; $\chi^2 = 1492$, $P < 0.0001$), considered individually.

Similarly, there was a significant effect of the interaction between genotype and time on fecundity (Figure 3b; $\chi^2 = 508.5$, $P < 0.0001$). In contrast, time alone (Figure 3b; $\chi^2 = 2836$, $P < 0.0001$), but not genotype alone (Figure 3b; $\chi^2 = 0.5706$, $P = 0.45$) significantly affected fecundity of RIL and CON wasps. Given that fecundity of CON lines was only higher than RIL lines for the first 48 h, we looked to see if there was a correlation between total sex ratio and early fecundity. Indeed, total sex ratio was significantly negatively correlated with fecundity in the first 48 h period (Figure 3c; $r_s = -0.4916$: $P < 0.0001$).

Finally, we looked at the total fecundity across individual RIL and CON lines, and found no significant difference in total fecundity after the 10-day period (Figure 4a; $\chi^2 = 0.3734$, $P = 0.5411$). There was however, a significant difference in fecundity between individual RIL

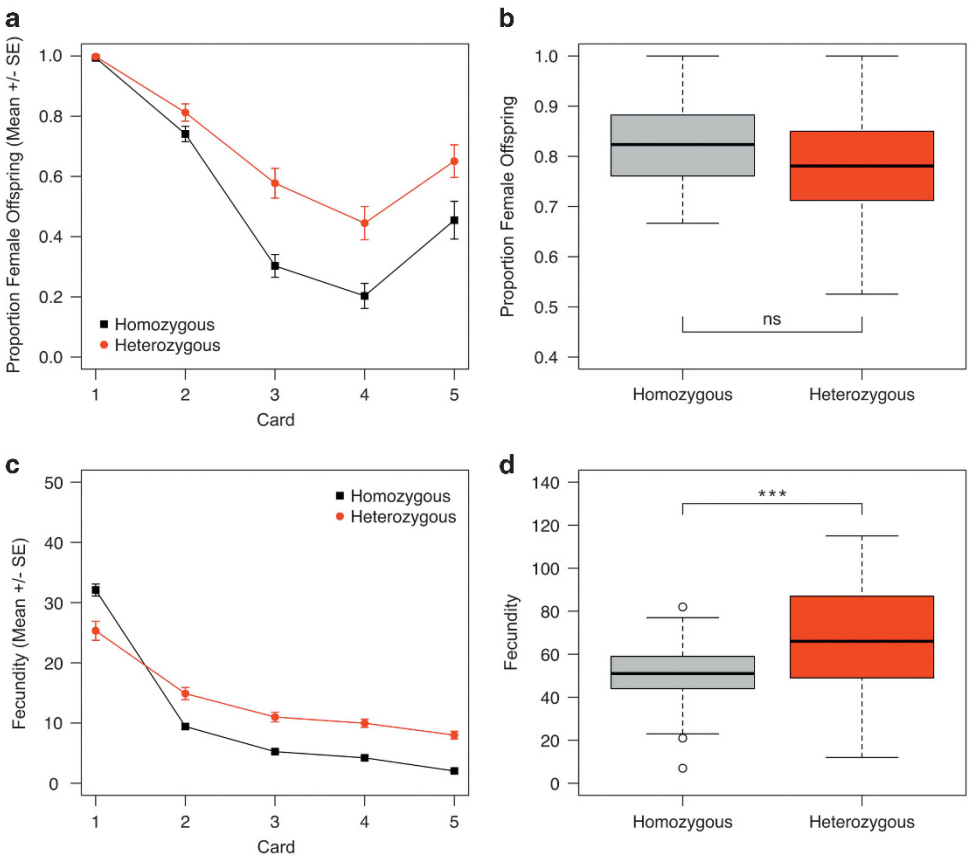

Figure 2 Sex ratios and fecundity of heterozygous wasps. 'Card' refers to a 48 h period. For example, card one is 0–48 h, card two is 48–96 h and so on. Sex ratio is denoted as the proportion of female offspring. (**a**) Variation in sex ratio across sequential 48 h periods. (**b**) Total sex ratio after the 10-day period. (**c**) Variation in fecundity across sequential 48 h periods. (**d**) Total fecundity after the 10-day period. Three asterisks represents $P < 0.001$.

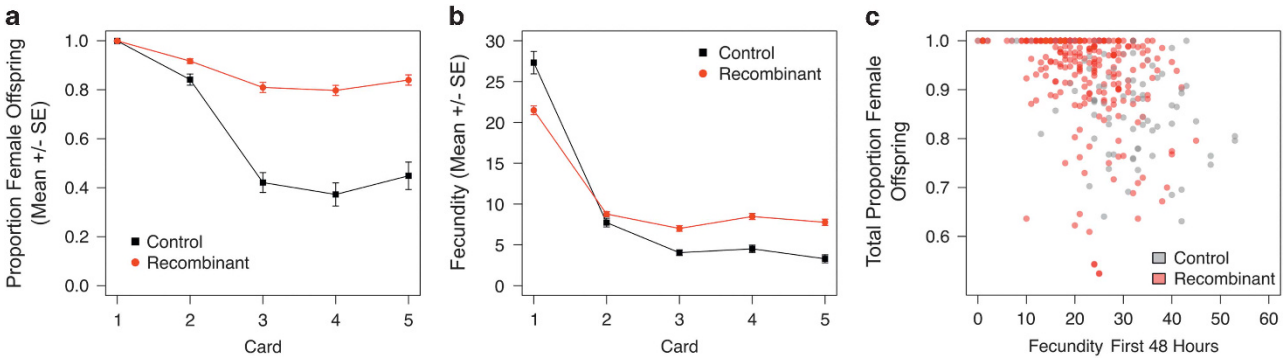

Figure 3 Relationship between fecundity and sex ratio of control and recombinant genotypes. 'Card' refers to subsequent 48 h periods and sex ratio is denoted as the proportion of female offspring. (**a**) Variation in sex ratio across sequential 48 h periods. (**b**) Variation in fecundity across sequential 48-h periods. (**c**) Correlation between fecundity in the first 48 h and total sex ratio. Points are semi-transparent, with darker color indicating more overlapping data points.

and CON lines, during the first 48 h (Figure 4b; $\chi^2 = 54.08$, $P < 0.0001$). Finally, total sex ratios produced after 10 days are significantly higher in RIL lines, as compared to the CON lines (Figure 4c; $\chi^2 = 5.3575$, $P = 0.0206$).

DISCUSSION

Parthenogenesis-inducing *Wolbachia* are unique in that their parasitic, selfish host-manipulations can result in the host becoming dependent upon the infection for reproduction (Zchori-Fein *et al.*, 1992; Dedeine *et al.*, 2001; Gottlieb and Zchori-Fein, 2001; Jeong and Stouthamer, 2005; Kremer *et al.*, 2009; Stouthamer *et al.*, 2010; Russell and Stouthamer, 2011). As host and symbiont become irreversibly linked, we might expect co-evolution of host and symbiont. Preliminary support for this hypothesis comes from finding that horizontal transfer of *Wolbachia* between *Trichogramma* hosts is rarely successful, and especially so for *Trichogramma* originating from populations completely fixed for infection. Either the infection will not establish in a new host, the novel infection fails to induce the parthenogenesis phenotype, or the infection is lost after a few generations in the novel host (Grenier *et al.*, 1998; Huigens *et al.*, 2004; Watanabe *et al.*, 2013). *Trichogramma pretiosum* is one such species where separate populations can either be sexual (Pinto, 1998), in rare cases mixed (Stouthamer *et al.*, 1990), or irreversibly asexual (Russell and Stouthamer, 2011), and have been shown to be resistant to horizontal transfer of *Wolbachia* (Huigens *et al.*, 2004). Here we can combine the nuclear genomes from *Trichogramma pretiosum* lines with these two different reproductive modes, and test how *Wolbachia* performs in the new system.

In asexual, completely clonal populations, outbreeding may be advantageous as a result of hybrid vigor. However, this could come at a cost for *Wolbachia* due to the breakup of co-adapted gene complexes. In our system, we see a heterozygote advantage, with no apparent cost for *Wolbachia* (Figure 2), which is congruent with previous studies showing that one complete copy of the maternal genome is sufficient for proper cyto-nuclear interactions (Breeuwer and Werren, 1995; Ellison and Burton, 2008). Heterozygote wasps produced more offspring without a reduction in female biased sex ratio, implying *Wolbachia* has no difficulty inducing parthenogenesis in the offspring of these hybrids. Given that zygosity has no impact on the total sex ratio or likelihood to survive, but heterozygosity results in higher fecundity, *Wolbachia* had a higher fitness when infecting heterozygous wasps.

We anticipated a breakdown in the F2 recombinant lines, with respect to the induction of parthenogenesis, due to incorrect interactions of nuclear and cytoplasmic genes that would start to appear when organisms are homozygous for paternal genes (Breeuwer and Werren, 1995; Ellison and Burton, 2008; Burton *et al.*, 2013). However, the recombinant lines produced similar numbers of total offspring with a more female-biased sex ratio than the control lines (Figures 3 and 4). Our data point to a high early fecundity driving this change in sex ratio. This corroborates recent findings that manipulating the rate of *Trichogramma* reproduction results in different sex ratios over the course of a week (Lindsey and Stouthamer, 2017). By producing fewer offspring early on, it appears that wasps can maintain sufficient levels of *Wolbachia* to provision to eggs, ensuring successful gamete duplication and thus the production of females. Our data from the recombinant and control lines indicate that the high early fecundity of control lines may be rapidly depleting *Wolbachia*, resulting in the quick drop in sex ratio (Figure 3). It is difficult to predict how these reproductive patterns might affect fitness in the field, but the slow-to-start recombinants may maintain an advantage if it results in sustaining a more female-biased sex ratio.

In contrast to our expectations, *Wolbachia* did not appear to have any difficulties inducing parthenogenesis in the new genetic backgrounds. This is especially curious given that the California *Trichogramma pretiosum* (our paternal line) seem to be resistant to infection with *Wolbachia*. If there were any strong level of adaptation to the Insectary line nuclear genome, we should be able to detect it after replacing ~ 50% of the genome. If there were a portion of the paternal genome that was incompatible with this *Wolbachia* strain, we should see it appear in at least a few of the recombinant lines (F2s), which would be homozygous for these alleles.

We think it more likely that *Wolbachia*-mitochondrial interactions are at play in this system. This is not surprising given the linkage of maternally transmitted cytoplasmic elements, and potential for mitochondrial sweeps due to *Wolbachia* infection (Turelli *et al.*, 1992). Unfortunately, it is difficult to disentangle *Wolbachia* and mitochondria in this system. Males are readily produced from asexual wasps with the help of antibiotics (Stouthamer *et al.*, 1990; Zchori-Fein *et al.*, 1992), and they can be mated to females from sexual populations. But our inability to horizontally transmit *Wolbachia* in this species is the limiting factor for creating new combinations of *Wolbachia* and mitochondria. Regardless, we can conclude that in this system there

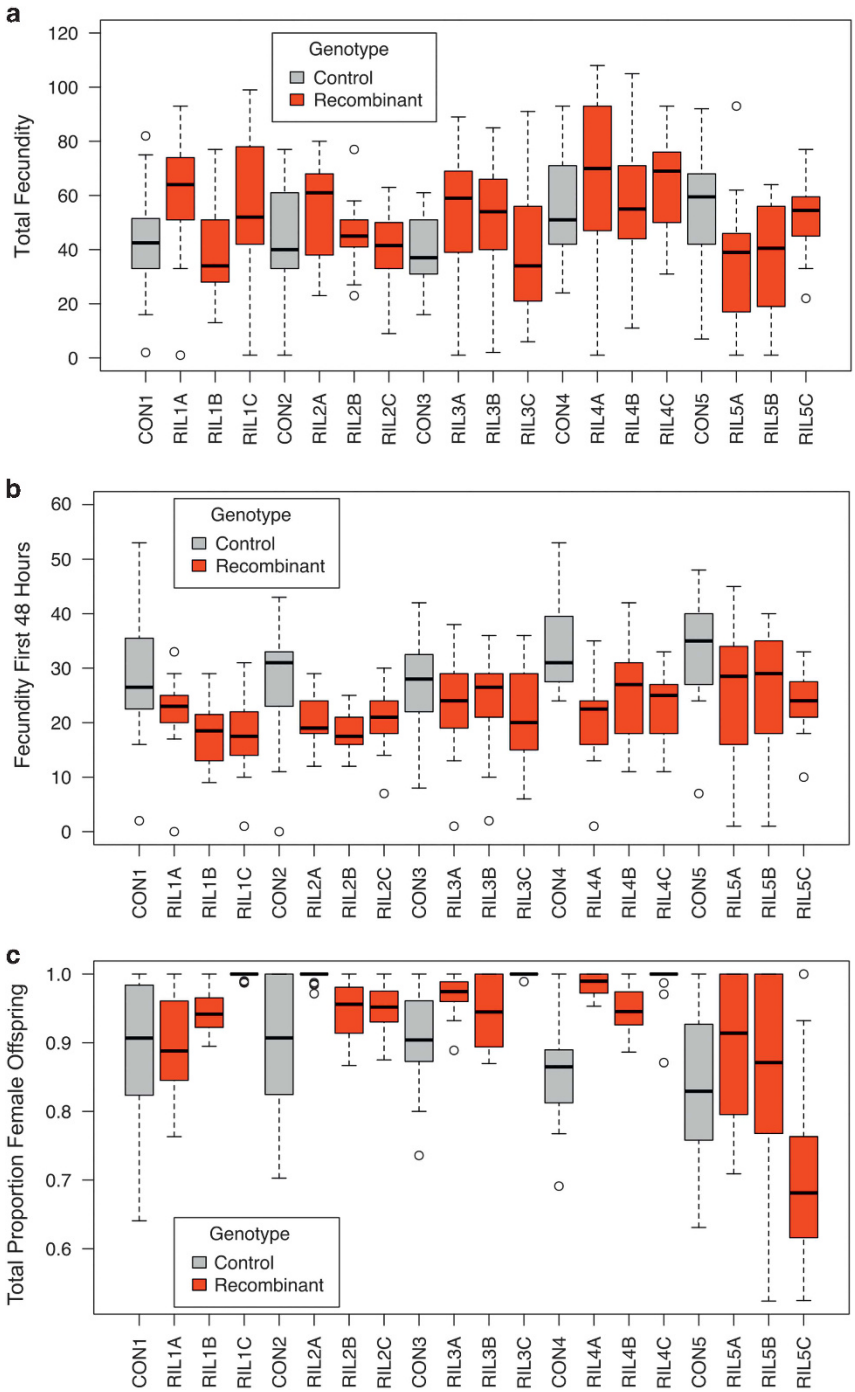

Figure 4 Sex ratios and fecundity for recombinant (RIL) and control (CON) isofemale lines. Open circles represent outliers and sex ratio is denoted as the proportion of female offspring. (**a**) Total fecundity for individual CON and RIL lines. (**b**) Fecundity in the first 48-h for individual CON and RIL lines. (**c**) Total sex ratios for individual CON and RIL lines.

is no evidence for co-evolution between *Wolbachia* and a particular host nuclear genome that once broken up results in a negative effect on the ability of *Wolbachia* to induce parthenogenesis, or in a reduction in the fitness of the *Wolbachia*-host combination. The interaction of *Wolbachia* and mitochondrial genomes beyond mitochondrial sweeps is likely an underappreciated feature of host-adaptation.

DATA ARCHIVING

All reproductive data from heterozygote, homozygote, recombinant, and control wasps are available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.j24c8.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (DEB 1501227 to ARIL) the United States Department of Agriculture (NIFA 194617 to RS and NIFA 2016-67011-24778 to ARIL); and Robert and Peggy van den Bosch Memorial Scholarships to ARIL. We thank Barbara Baker and Christina Luu for their assistance in counting *Trichogramma*, Sarah Lillian for statistical advice, and Paul Rugman-Jones, Eric Smith, Matthew Daugherty, and Jason Stajich for discussions and feedback on drafts of the manuscript.

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